TECHNOLOGY FEATURE

THE CLINICAL CODE-BREAKERS

DNA sequencing is helping clinicians to unravel the underpinnings of disease in individual patients.

ROBERTS INDIVIDUALIZED MEDICAL GENETICS CENTER/CHILDREN'S HOSPITAL OF PHILADELPHIA

The team at the Roberts Individualized Medical Genetics Center helps to turn sequence data into meaningful clinical information.

BY MICHAEL EISENSTEIN

For most of the day, the hallway housing the Division of Genomic Diagnostics at the Children's Hospital of Philadelphia (CHOP) in Pennsylvania is empty and peaceful, marred only by the faint hum of equipment and occasional scraps of conversation. But at 10:15 every morning, it fills with a bustling group of clinicians, bioinformaticians, geneticists, counsellors and administrators, who huddle around a pair of information-packed displays. Over the next 15 minutes or so, this diverse team will hash out any technical or logistical obstacles that could impede its daily goal: delivering accurate genomic data to guide the diagnosis of children with severe and potentially deadly diseases.

Even division chief Nancy Spinner is taken aback by how the team has blossomed. "It's hard to describe all of the changes that have happened," she says. "Since we started, we've certainly more than doubled in size." The team's work represents the clinical evolution of a 2011 research study called PediSeq, which was headed by Spinner and her husband, clinical geneticist Ian Krantz. Spinner says that Krantz had grown exasperated with the limited success of single-gene tests, which required skilful matching of symptoms to known disease genes — and a healthy dose of luck. "He said, 'We need to change the way we do genetics,'" recalls Spinner.

Clinical sequencing services have now flourished in several centres around the world. They have generally evolved organically from pilot studies that aimed to explore the clinical utility of genomes or exomes — the 1% of the genome that codes for proteins.

For diseases with a well-established genetic foundation, such as cancer and some developmental disorders, sequencing can be a game-changer. "We've found that for children under two, one-third of those that got a diagnosis had some change in their clinical management," says Clara Gaff, executive director for the Melbourne Genomics initiative in Australia.

Accordingly, uptake is skyrocketing. CHOP has already sequenced 300 exomes this year — and that is nearly twice as many as it sequenced in the whole of 2015. Similarly, pioneering work in speeding up the diagnosis of severely ill newborns by paediatric genomics researcher Stephen Kingsmore and his colleagues has led to a high-powered clinical initiative at Rady Children's Hospital in San Diego, California. "We're performing rapid genome sequencing in about 200 kids a year out of our own intensive-care units," he says.

Yet for all the excitement, a sequencing-based diagnosis is far from a sure thing, and many patients receive results that yield no clear insight. Plus, medical sequencing centres have to grapple with serious technical and medical ▶

challenges, not to mention proving that the pricey programmes can deliver a cost-effective diagnostic solution.

A GOOD START

An estimated 6–8% of children are born with a developmental disorder that has a genetic origin. Many of those disorders arise from mutations in a single gene, making them an excellent match for a sequencing-based 'dragnet' search to identify the culprit. Sometimes the resulting discovery empowers clinicians to contain the damage. For example, the team at CHOP used targeted exome analysis to link a 9-year-old patient's hearing and vision problems to a mutation in a gene that regulates the metabolism of riboflavin, which can lead to severe neurodegeneration. Riboflavin supplementation prevented further decline, Spinner notes. A younger sibling also tested positive for the mutation, Spinner adds, "so they could start earlier there".

Such clear-cut successes are rare, but geneticists routinely identify causative mutations for 20–30% of hereditary disorders. Even if the findings aren't directly actionable, they can comfort the family and inform medical care. For instance, Jenny Taylor, co-director of the tranlational genomic medicine programme at the Wellcome Trust Centre for Human Genetics in Oxford, UK, says that her team pinpointed the genetic basis for an inherited kidney disorder — a finding that could help in the identification of family members who might be at heightened risk for needing a kidney transplant.

Whereas early research efforts focused on people with well-studied developmental problems, clinical sequencing centres are starting to widen their scope. "We audit admissions every day in our intensive-care units," says Kingsmore. "If there's a child who might benefit from a genome sequence, we'll do it."

Most of the tests currently used in clinics are targeted surveys that use a 'capture' step to isolate the exome. Heidi Rehm and her colleagues at the Laboratory for Molecular Medicine at Partners Personalized Medicine in Boston, Massachusetts, routinely analyse exomes to diagnose genetic disorders. They sequence the entire exome, but initially analyse only targeted gene panels to save money and time. They look at the rest of the exome data only if those panels come back negative.

Whole-genome sequencing (WGS) captures information that might be overlooked in exome analyses, but only a handful of clinical centres routinely use the method. One of those is the Wellcome Trust centre. "There were a lot of unsolved cases with exome [sequencing], and we were not getting traction on the non-coding and regulatory regions," Taylor says. However, genetic variants in those regions can be hard to interpret, and WGS creates a considerable data burden. Individual genomes can contain millions of variants, the vast majority of which will not be linked to the disorder in question.

Even for exomes, diagnosis is a painstaking process. Any given exome might contain tens of thousands of nucleotide changes relative to a healthy reference, and every one of those needs to be compared against databases such as ClinVar — a global repository that combines gene data with clinical information and an assessment of likely pathogenicity — and gnomAD, a collection of some 120,000 exome sequences that indicates how common a variant is. A rare disease will almost certainly arise from a rare variant, and it is important to have a diverse pool of data to eliminate biases from ethnic or geographic genetic variation.

Many labs sequence 'trios' — the patient and both parents — to eliminate benign differences that are present in healthy family members. Comparing the sequence with those in population databases can filter out more than 95% of the changes, says Livija Medne, co-director of the Roberts Individualized Medical Genetics Center at CHOP — the clinical partner that attempts to translate the variant data from Spinner's team into a diagnosis, "but you're still left with a couple of hundred to sort through, and having trios is extremely helpful".

Ultimately, there's no substitute for clinical expertise, and all the data are typically carefully reviewed by panels of clinicians to verify that any suspected causative mutations are a realistic diagnostic 'hit'. This can take months, but Kingsmore has demonstrated that a streamlined diagnostic pipeline combined with smart bioinformatics can accelerate the process considerably. "In practice, our fastest routine genome test takes about two days," he says. Setting up such a high-speed workflow is no mean feat, and Rady is now offering its services to other paediatric hospitals. "We've put into operation a plan to share this with every paediatric and neonatal intensive-care unit in the world," says Kingsmore.

TAMING TUMOURS

Molecular genetics is also transforming cancer care, as oncologists try to identify individualized treatments that might kill tumours according to their mutational profile. Many leading cancer centres now offer tumour sequencing services, and clinicians are eager to take advantage. "We've enrolled or run our test on over 3,000 patients," says Arul Chinnaiyan, director of the Michigan Center for Translational Pathology in Ann Arbor, which offers the exome-based MI-ONCOSEQ test. "And I would say that in upwards of 90% of cases, we find what we think are the biological drivers of the tumour."

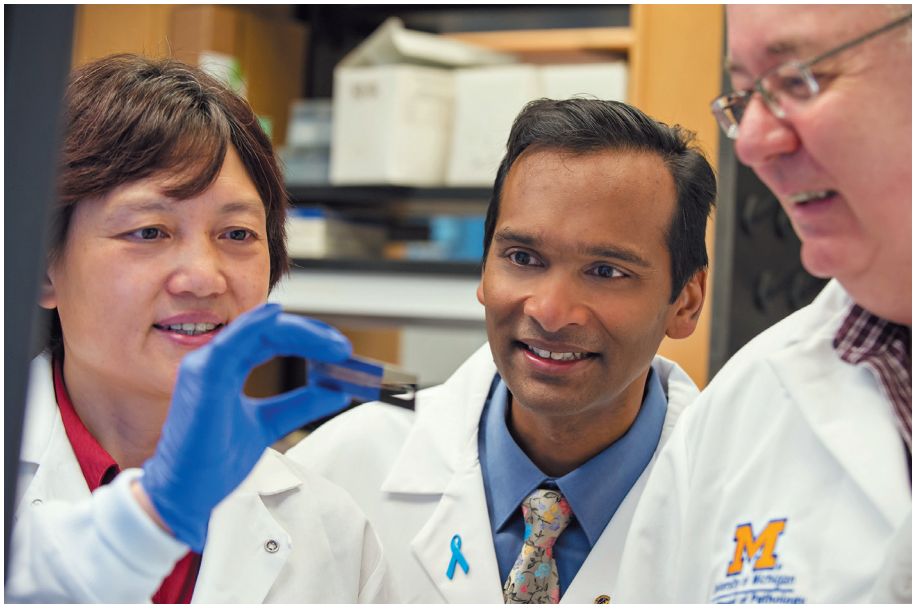
Cost control

Jenny Taylor cringes when she hears the expression '$1,000 genome'. "That is not anywhere close to the price that we get as a smallish facility in Oxford," says Taylor, who is at the Wellcome Trust Centre for Human Genetics in Oxford, UK. Although the price can indeed dip below US$1,000 in a production-scale facility, most centres spend several times that. Indeed, exome- or genome-based diagnoses can exceed $5,000 and $24,000, respectively.

Just setting up a centre is a huge investment, especially with top-of-the-line instrumentation. "These are million-dollar machines, and it's hard for hospitals to pay for them," says Christian Marshall, co-director of the Centre for Genetic Medicine at the Hospital for Sick Children in Toronto, Canada. Rady Children's Hospital in San Diego, California, managed to achieve record-breaking sequencing times, but with an outlay of $10 million in equipment and computing infrastructure, notes director Stephen Kingsmore. Laboratories that have heavy diagnostic traffic can justify these cutting-edge machines — but only when they run at capacity. "You have to have the volume to support these higher-throughput instruments," says clinical geneticist Heidi Rehm at Partners Personalized Medicine in Boston, Massachusetts, which now outsources its genome sequencing to the nearby Broad Institute.

Expertise isn't cheap either, and data analysis is a major wild card in the cost of testing. Some exomes contain 1,000 candidates, which must be carefully winnowed down for a diagnosis, says Nancy Spinner, head of genomic diagnostics at the Children's Hospital of Philadelphia in Pennsylvania. "All in all, hands-on time can range from 3 to 16 hours," she says. It requires a team of well-trained (and well-paid) experts. Cost-cutting on one end can create work at the other. Take for instance, the use of 'trios', in which the exome of a patient is compared against those of their parents. "It's three times the cost to do the sequencing," says Marshall, "but it actually saves you a ton of time on the analysis side."

These high and unpredictable costs pose a challenge for both private-sector healthcare payers and national health systems as they strive to assess the cost-effectiveness of genomic screening. According to Clara Gaff, executive director of the Melbourne Genomics initiative in Australia, the key is to play to sequencing's strengths. For example, she and her colleagues have shown that using exome diagnostics can triple the diagnostic power at one-third of the price of conventional techniques used to diagnose young children with genetic developmental disorders. "It's cost-effective when used early in the diagnostic pathway and replaces tests that are no longer necessary," she says. M.E.


UNIV. MICHIGAN ROGEL CANCER CENTER

Arul Chinnaiyan (centre) directs the Michigan Center for Translational Pathology.

In some ways, identifying mutations that are unique to the tumour is easier than hunting down those that cause inherited disorders. Oncologists have assembled an impressive roster of genes that are known to trigger uncontrolled growth and invasion when mutated, and some centres — including CHOP — are having great success with panel-based analyses of known trouble spots. "Over 90% of our positive tests have either diagnostic or prognostic significance," says Marilyn Li, CHOP's director of cancer genome diagnostics.

That said, the mutations underlying cancer are often more complicated than the single-base mutations commonly seen in developmental disorders. Cancer can occur when genes are duplicated, deleted or spliced onto unrelated genes, often as a result of damage to the chromosomes. These structural changes are detectable with targeted gene panels or exome-based tests, but can be captured more reliably with WGS. "WGS is incredibly powerful for structural rearrangements, including chromosomal translocations and inversions," says Sharon Plon, a geneticist at Baylor College of Medicine in Houston, Texas.

And unlike hereditary disorders, in which mutations are present throughout the body, tumours can be highly heterogeneous, with cancerous and healthy cells intermixed, and extensive genetic variation even within a tumour. This means that more sequencing reads must be obtained for a given region to confirm that a variant is real, and that bumps up the cost. A growing number of labs are also coupling exome and RNA analysis to detect gene products that are defective or produced at inappropriate levels. "It's a more reasonably priced way to try to provide more direct analysis of the effect of variation on gene expression," says Plon.

Such analyses can affect patient care in multiple ways. There might be a drug available that targets the mutation in question, for instance. "I would say that maybe 30–40% of our cases end up being clinically actionable," says Chinnaiyan. In many cases, the only treatments available for a given mutation are experimental ones, and that means a "clinical trial has to exist and the patient has to be able to get into it". Mutational profiling can also correct diagnoses — and thereby prognoses — that were made on the basis of the pathology of the tumour and turned out to be inaccurate. "One of our patients had an improved prognosis, and therefore chose to have radiotherapy rather than chemotherapy," Taylor says.

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A GROWING CROWD

The workhorse of clinical sequencing is the HiSeq 2500 machine, from Illumina in San Diego, California. This instrument can produce a full genome sequence in 27 hours — an impressive feat, but not necessarily sufficient for the demands of many clinical sequencing centres.

In 2017, Illumina increased its capacity with the NovaSeq, which produces a lot more data, more quickly. "It can decode up to 6 genomes in 15.5 hours," says Kingsmore, whose team recently began working with the instrument. But with a price tag of nearly a US$1 million, not including reagents and routine maintenance, NovaSeq is a heavy investment. Even the cheaper units can strain hospital budgets (see 'Cost control').

The human element remains a major bottleneck in the race for a diagnostic result, but Li's team is using machine-learning-based approaches that get smarter with each new data set they inspect — the algorithms learn how to differentiate signal from noise within highly mutated cancer genomes. "This allows us to filter out 70% of our variants so that we don't have to manually look, increasing our efficiency drastically," she says. And by coupling NovaSeq to a turbocharged artificial-intelligence platform for analysing medical records and using top-of-the-line computer hardware, Kingsmore and his team have managed to achieve a record-setting 19.5 hours from sample to answer.

Nevertheless, many diagnostic attempts still end in disappointment. As impressive as a 50% 'hit rate' might be, it still means that half of the patient population goes home empty-handed, and for many categories of genetic disorders, that could rise to 70–80% . Gaff notes that this uncertainty is not new for clinical geneticists — they have been grappling with ambiguous results since the early days of genetic testing for breast cancer, in the 1990s. "Managing clinician expectations is the critical thing," she says. "With genomic testing, we see some huge enthusiasm which may not always be warranted, as well as scepticism that also isn't warranted."

But with more data comes clarity. A study published earlier this year showed that follow-up analysis one year later yields a diagnosis in 11% of previously unresolved clinical exome cases (L. J. Ewans *et al. Genet. Med.* https://doi.org/10.1038/gim.2018.39; 2018), and Taylor's team is among those performing routine reanalysis. "We never consider a case closed," she says. And as eager patients queue up for analysis, clinical genomicists are keen to help where they can. "I personally think that every cancer patient should have their tumour sequenced, if the price is right," she says.

Clinicians are now setting their sights on other widespread disorders with complex origins, such as diabetes or cardiovascular disease. Such diagnostic capacity is currently out of reach, but research efforts are exploring the benefits of genomics in the broader community. Christian Marshall, co-director of the Centre for Genetic Medicine at Toronto's Hospital for Sick Children, points to efforts such as the UK Biobank, which is collecting vast amounts of biomedical data — including, in many cases, exomes — from 500,000 volunteers to identify possible predictors of long-term health and disease. "Once you start sequencing hundreds of thousands of people and have some phenotypic data layered around it, then it becomes possible to try and determine how we can use genomics in general health," he says.

Beyond technical ability, Medne thinks that society as a whole will need time to move into this era. It will also need a better understanding of what it means to be at risk of developing or transmitting a hereditary disease, and to develop better legal protections against potential discrimination. "We need to get to where genomics is a part of health care," says Medne, "as opposed to now, where it's a part of disease." ■

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CORRECTION

The Technology Feature 'The clinical code-breakers' (*Nature* **562**, 291–293; 2018) gave the wrong affiliation for Jenny Taylor. She is co-director of the translational genomic medicine programme at the Wellcome Trust Centre for Human Genetics.